sartorius stedim biotech

Operating Manual

BIOSTAT® CultiBag RM 600

85034-539-09

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Introduction

This operating manual describes the installation and operation of the bioreactor BIOSTAT® CultiBag RM 600.

Disclosures

The bioreactor BIOSTAT® CultiBag RM 600 has been designed for the development and optimization of processes for the cultivation of cells or microorganisms in disposable systems with low shear.
The safe operation of the bioreactor assumes special knowledge in aseptic techniques for the safe handling of the microorganisms or cells.
In order to prevent possible biohazards the cultures of microorganisms and cells may require special handling and waste disposal considerations for the equipment and for disposal of the culture contents and disposable bag bioreactors.

This operating manual contains appropriate safety notes only on possible hazards and remedial measures if they apply to the users of this equipment and its associated equipment. Such safety notes are supplementary to regulations distributed by the company or local authority for the process. It does not refer to conditions and regulations on the protection of the staff and the work environment, whether they are legal or binding in any way.

The production scale disposable bioreactor system BIOSTAT® CultiBag RM 600 is part of the product range of Sartorius Stedim Systems GmbH.

If you have questions on the BIOSTAT® CultiBag RM 600 or further products of Sartorius Stedim Systems GmbH, please contact:

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Release Notes

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Legend

BIOHAZARD This symbol indicates process dependent risks for the staff.

Possible hazards, either damages of equipment or effects on personal health, will depend on the process and the microorganisms or cells applied.

This symbol indicates possible, either damages of equipment or effects on personal health caused by electricity.

NOTICE indicates property damage to the equipment.

This symbol indicates steps of operation which must be executed with special care or at which important aspects need to be considered, e.g. in order to avoid erroneous operation or malfunctions.

☐ The “button” indicates other noticeable information.

1., 2., .. Numbered paragraphs show steps of operation, which need to be processed in the order, as indicated.

This symbol refers to secondary information, which is given in this or in other documentations.

General Safety Notes

Organizational Issues
(legal issues, staff & working place)

The enterprise (i.e. the responsible authorities) shall ensure that the required conditions appropriate for the process are provided:

1. The personnel shall be qualified for the process, for which the bioreactor is applied, they shall know the hazards possible, and carefully familiarize themselves with the handling of all equipment, which is associated with the bioreactor.

BIOHAZARD

[refer e.g., to
"Legal regulations on
biological safety"]

2. The access to the work place must be restricted for persons not authorized to work with the bioreactor. If the process can cause hazards, e.g. resulting from the culture or media, the work place shall be marked by appropriate safety labels, such as "BIOHAZARD", and be isolated by appropriate barriers.

3. The work place shall be suitable for the process, i.e. be resistant against acids, alkaline solutions or the media. In the case of contamination, e.g. by the culture, it shall easily be possible to decontaminate, disinfect and clean the work place.

4. The enterprise (i.e. the responsible authorities) must issue safety regulations about hazards which can occur in the process at the work place for the staff and work environment. Required safety facilities have to be provided.

5. In general, the staff shall wear suitable working clothes and personal protective equipments (e.g. gloves, safety goggles as well as respiratory protection, if required).

Installation at the working place

The working place and the utility supplies shall fullfill the requirements according to the specifications of the bioreactor.

2. Provide that the laboratory mains and the supplies of gasses fulfil the specifications of the bioreactor.

3. Only use equipment and peripheral devices which Sartorius Stedim Systems has released for the BIOSTAT® CultiBag RM 600.

4. Tighten all connections of the laboratory, of the CultiBag RM 600 and the peripheral devices especially carefully. Test for leaks and unintentional release.

5. Ensure that all equipment and the peripheral devices of the bioreactor are in good condition. This is especially true for the CultiBag RM 600. Do not use damaged parts (even if damages are only suspected).

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1. Structure and Function

1.1 Usage of BIOSTAT® CultiBag RM 600

The BIOSTAT® CultiBag RM 600 is a large scale rocking platform for cultivation of cells and microbes in Disposable Bag Bioreactors (CultiBag RM 600). The Rocking platform is combined with the BIOSTAT® RM Control Tower which allows monitoring and control for pH, DO, temperature, rocking angle and rocking rate.
BioPat® MFCS | DA software for external data logging is included.

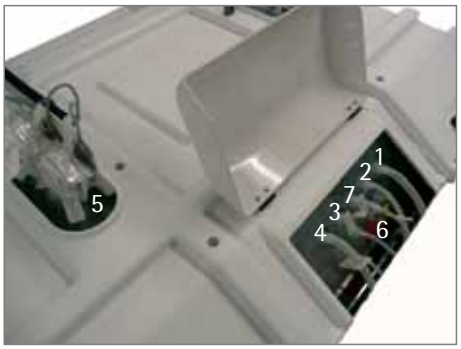
Rocking motion mixing technology utilizes mechanical energy to ensure homogeneous mixing of cells with low shear. Energy input is effected by rocking the CultiBag RM 600 side to side, generating a fluid movement in the cell culture and medium.
In this way the surface of the medium is continuously renewed for bubble-free surface aeration.
CultiBag RM 600 disposables bag for cultivations of up to 300 L of culture volume.

Fig 1: Set Up of BIOSTAT® CultiBag RM 600 optical

The rocking platform, Rocker 600, is operated via the Control Tower.
All set points and control functions will be controlled and displayed in the BIOSTAT® RM Control Tower.
For further information regarding connection of the Rocker 600 to the BIOSTAT® RM Control Tower see associated manuals for Control Tower.

1.2 CultiBag RM 600

Cell cultivation is performed inside the CultiBag RM 600 disposable bioreactor chambers, which are specially designed to work with the BIOSTAT® CultiBag RM 600. These bags are delivered presterilized and ready for use. The CultiBag RM 600 is produced of multilayer film, USP class VI and is designed for single use operations. This feature eliminates the need for cleaning and sterilizing the bioreactor with considerable savings in labour, no danger of cross-contamination and greater assurance of sterile operation.



CultiBag RM 600L optical, mounted on table

1 Fill port, C-Flex tubing with MPC coupling
2 Spare port with luer connector
3 Sample port, with luer-septum for sampling or inoculation
4 Fill | drain port, C-Flex tubing with Luer
5 Air outlet sterile filter
6 pH and DO probes
7 Fill port, C-Flex tubing with MPC coupling

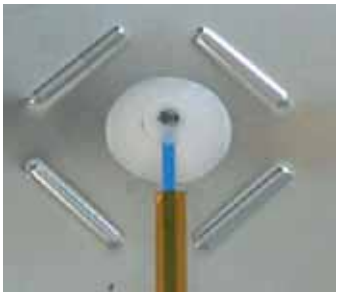
CultiBag RM 600L only are recommended for use with the BIOSTAT® CultiBag RM 600.

1.3 System Structure

The bioreactor BIOSTAT® CultiBag RM 600 is compiled from the following components:

☐ Rocker 600

☐ BIOSTAT® RM Control Tower 600 and the hexagonal frame



☐ Surface Temperature Sensor DS600L-RMPT

☐ 2× Filter Heater for BIOSTAT® CultiBag RM 600 DS600L-RMFH

☐ Aeration tubes labelled
☐ Quick Installation Guide hard copy

☐ CD ROM including User Manual and Quick Installation Guide

CultiBag RM is not a part of delivery!
Please specify and order separately.
For further information please contact your Sartorius Stedim Biotech representative.

1.4 Technical Data

1.4.1 Operation

The BIOSTAT® CultiBag RM 600 is a fully automated, PLC controlled system.
Operation is via BIOSTAT® RM Control Tower.

1.4.2 Gassing module

The BIOSTAT® CultiBag RM 600 contains a superior gassing system, which consists of 4 rotameters (Air, O_2, N_2, CO_2), 1 mass flow controller for total flow and 1 mass flow controller for CO_2.
Flexible hose with connectors to connect CultiBag RM with BIOSTAT® RM Control Tower 600 are included.

Mass flow controller (MFC 1) 0.4-20L for air, O_2, N_2
Mass flow controller (MFC 2) 0.2-3 L for CO_2

1.4.3 Rocking rate

2...16 ± 1 rocks/min, adjustable at BIOSTAT® RM Control Tower.

1.4.4 Rocking angle

4...10°, ± 0.5°, adjustable at BIOSTAT® RM Control Tower.

1.4.5 Temperature control

PLC programmed PID controller built-in the BIOSTAT® CultiBag RM 600.
Set temperature is adjusted at BIOSTAT® RM Control Tower.
Cooling by ambient air only. No cooling device required.

An electrical resistance heater plate is installed below the rocking table with a built-in safety temperature switch.

Heating power: One section with 650 W.
Temperature range set temperature +15...+44°C, ± 0.1°C.

1.4.6 Measurement of DO and pH

Two reusable non invasive optical fibres for pH | DO are inserted into the respective sensor ports of the CultiBag RM 600. The optical fiber transmits light of specific wavelength, to the sensor patch and returns the luminescence response from the sensor back to the measuring amplifier. The light fibre cables are marked with colours to avoid insert into the wrong port of the CultiBag RM 600.
– DO (yellow)
– pH (red)
For further information please refer to BIOSTAT® RM Control Tower manual.

1.4.7 Communication

Ethernet connection in order in connect e.g. BioPAT® MFCS/Win or BioPAT® MFCS/DA.

1.4.8 Materials of Construction

Electrical cabinet and frame, housing BIOSTAT® RM Control
Tower 200: Stainless steel 1.4301 (304), brushed, electropolished.
Rocking table: Stainless steel 1.4435, brushed, electropolished.
Lid: ABS with UV layer.

1.4.9 Ingress Protection

IP 43 Minimum.

1.4.10 Dimensions | Weight

Dimension with mounted BIOSTAT® RM Control Tower :
– Rocking table in horizontal position: W × D × H 1.97 × 1.47 × 1.33 m.
– Weight without CultiBag RM 600: 340 Kg.

1.4.11 Power supply

Only one power supply for the BIOSTAT® CultiBag RM 600 is available: 3 × 400VAC, +N, +PE, 50-60Hz, 16A

1.4.12 Ambient temperature range

0...+50°C.

1.4.13 Characteristics, Specification of CultiBag RM, operation modes RM Control Tower

CultiBag RM 600L	Number of bags mounted	Min. working volume* in CultiBag RM 600 [L]	Max. working volume in CultiBag RM 600 [L]
1× CultiBag RM 600L	1	60 L	300 L

* CultiBag RM 600L with integrated disposable sensors pH and DO require higher min. working volume.

1.5 Surface Temperature Sensors

PT100 is a resistance thermometer class A (EN60751), 3-wire, which measures the temperature of the CultiBag RM 600.

It is plugged into the BIOSTAT® CultiBag RM 600 with a LEMO plug, and placed under the bag in the middle of the rocking table.

1.6 Filter Heater

Heating of CultiBag RM 600 generates small amounts of condensation rising into the air outlet filter. The filter heater keeps the vent filter dry and prevents plugging of the filter media. A green LED shows the heater is heating. The filter heater is placed at the lid. Magnets are used for easier fit.

Temperature range: 35...50°C, ±5°C
Heating power: 24V, 6W

2. Delivery and Installation

2.1 Delivery Checks

The BIOSTAT® CultiBag RM 600 is delivered after careful functional tests. Should there be any damage in transit or if the system is inoperable after installation due to damaged and defective component parts, please follow the notes below.

1. Check the order confirmation with the delivery documents according to the order placed.
2. Check all units and component parts for damage.
3. Report missing parts or damage in transit to your local Sartorius Stedim Biotech Representative.
4. Check the proper function of all units and component parts before the first cultivation process.
5. Record malfunctions carefully. Return the claim report to your local Sartorius Stedim Biotech Representative.

2.2 Space Requirements

The table below details the space requirements of bioreactor BIOSTAT® CultiBag RM 600 units:

Space requirements for	W × D [m]:
BIOSTAT® CultiBag RM 600	2.5 × 1.5
Operator needs in minimum at front side	Recommended: approx. 1.5 m
For installation | maintenance | calibration | repair around the unit	Recommended: approx. 1.0 m

Please verify your door opening is in minimum 1.5 m wide to transport the system into your operation room.

If BIOSTAT® CultiBag RM 600 RM is moved on its wheels; take its weight into consideration. Take precautions if moved up or down on a ramp and make sure the ground is strong enough to support the weight.

2.3 Connection of the Supplies

Required fixtures and fittings are enclosed in the delivery. Additional connection fittings for special requirements are available; please contact your local Sartorius Stedim Biotech representative for details.

2.3.1 Preparing the Work Place

1. During set up and installation of the bioreactor ensure that sufficient workspace is available: (See section space requirements).

☐ Placement of the BIOSTAT® CultiBag RM 600L
☐ Connection to the facilities
☐ Placement of the CultiBag RM 600L
Additional peripheral devices.

2. Check the mains connection:
☐ Mains supplies in at the work place must fit to the specifications of the bioreactor.

2.3.2 Mains connection

CAUTION Risk of voltage damage to equipment!

The mains supply must fulfill the specifications of the units.
Voltage fluctuations must not occur.
Verify that correct and interference free mains supply is available.
Never connect to wrong mains specifications.
Interrupt power supply before opening the cover of electrical cabinet.

Check the hardware labels to determine whether the correct mains version is supplied and the power cords are equipped with the plugs required for the supplies.
If any of the units have incorrect mains version or power cords have wrong plugs, please contact your local Sartorius Stedim Biotech service person.

The BIOSTAT® CultiBag RM 600 is verified for electrical security from plant side.
It is still necessary for customer to check the electrical security and electrical compatibility of the internal power mains.

2.4 BIOSTAT® CultiBag RM 600

2.4.1 Connections, Interfaces

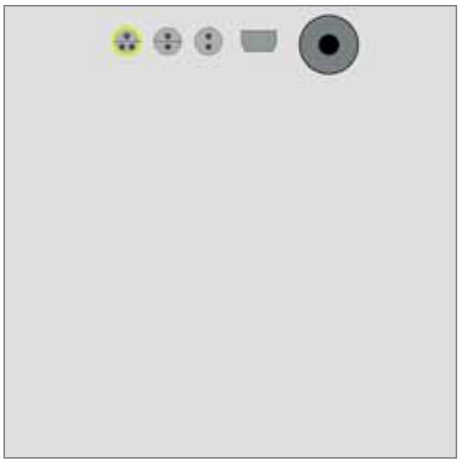


Fig 2: BIOSTAT® CultiBag RM 600: Schematic rear side view of electrical cabinet

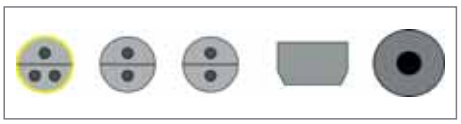


Fig 3: BIOSTAT® CultiBag RM 600: Schematic view of the sockets

Connectors and switches

1 Socket to connect surface temperature sensor red
2 Socket to connect filter heater
3 Socket to connect filter heater
4 Socket D-Sub RS232 to connect BIOSTAT® RM Control Tower
5 Socket to connect BIOSTAT® RM Control Tower

2.4.2 Safety

The area below the rocking table is protected by a laser scanner. It stops the rocking motion immediately when something enters the hazard area.

For safety reasons you must switch off the system BIOSTAT® CultiBag RM 600 before restarting the rocking motion after an Emergency stop.

Attention! Do not touch the area showed in the picture at any time! It is not protected by the laser scanner and will cause serious injury.

Attention! The yellow-black hazard area at the backside of the plant is not protected by the laser scanner. Do not touch the running unit.

3. Getting Started

3.1 Overview

The commissioning of the BIOSTAT® CultiBag RM 600 and its operation for the respective process covers the following steps:

1. Transportation, unpack equipment.
2. Equipping the system
3. Preparation and inflating of the CultiBag RM 600L
4. Cultivation
   - Preparing for the cultivation
   - Sampling and Inoculation
   - Media exchange
5. End of the process and putting out of service safely
6. Cleaning and maintenance work

3.2 Installation Kit

The scope of the delivery includes a kit for the required fittings and connections.

1. Use only fixtures and fittings supplied by Sartorius Stedim Biotech and released for use with the BIOSTAT® CultiBag RM 600.
2. Replace damaged components and spare parts by components delivered or released by Sartorius Stedim Biotech.

Malfunctions caused by the use of parts not approved for use with the BIOSTAT® CultiBag RM 600 are not covered by warranty.

3.3 Transportation, Unpack Equipment

3.3.1 Transportation

1. If BIOSTAT® CultiBag RM 600 is moved on its wheels; take its weight into your consideration. Take precaution if moved up or down on ramp and make sure the ground is strong enough to support the weight.

2. If lifted by fork lift or pallet truck, please ensure BIOSTAT® CultiBag RM 600 is lifted up by its frame. Lift up at rear or front side. Lift up at side towards control cabinet. If required, fix at fork. Please ensure you do not damage cables.
   Only lift with fork lift or pallet truck. Please do not use chain blocks or similar device. Please lift only at the lower frame.

3. BIOSTAT® RM Control Tower is mounted at pallet. Only transport with fork lift or pallet truck.

4. Keep equipment dry while transportation or storage.
   Do not lift at the rocking table or at the control cabinet.
   Note the weight and the dimensions of all devices. Use appropriate transport carriers.
   Do not lift the Rocker 600 with the BIOSTAT® RM Control Tower mounted and | or with liquid containing CultiBag RM inside.

3.3.2 Place at Final Destination

1. Move the BIOSTAT® CultiBag RM 600 to the final place where it will be installed. Lock all wheel breaks. Please make sure the rocking table is placed in horizontal position on an even surface.

Take care when moving onto inclined plane. Take it's heavy weight into your consideration. Do not move the BIOSTAT® CultiBag RM 600 mounted with liquid containing CultiBag RM inside.

3.3.3 Unpack Equipment

1. Remove the carton closure from BIOSTAT® CultiBag RM 600. Sink the BIOSTAT® CultiBag RM 600 carefully from the pallet on a horizontal floor. A fork lift may be used. Lift only at the lower frame. Check that the equipment is intact and complete.

2. Unpack BIOSTAT® RM Control Tower carefully. Avoid any damage to front mounted air flow meters and connectors.

3.3.4 Room of Installation

1. The operator needs only access at the front of the BIOSTAT® CultiBag RM 600. For calibration and maintenance, access from the rear side and left side is also required.
   The BIOSTAT® CultiBag RM 600 is mobile, but must not be moved with a filled bag inside.

NOTICE

2. Place the BIOSTAT® CultiBag RM 600 on a horizontal, safe and stable floor.

NOTICE

3. Do not place any equipment close to the rocking table to avoid any blockage or damage. Also do not place the BIOSTAT® CultiBag RM 600 to jut out from parts of wall which could be a hazard risk.

4. The measurement of pH and DO is based on chemical sensors. The chemical pH sensor is susceptible to photo bleaching, keep it covered. Avoid intensive light such as sun beam shining on the BIOSTAT® CultiBag RM 600 with mounted CultiBags.

   Do not expose the Cultibag RM on direct sun beam.

3.4 Equipping the system

3.4.1 Mount BIOSTAT® RM Control Tower

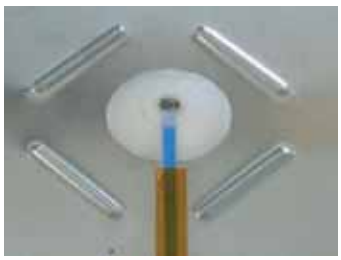
1. Connect the BIOSTAT® RM Control Tower with BIOSTAT® CultiBag RM 600 using included RS232 connection cable and power supply.

NOTICE

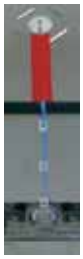
Do not modify the BIOSTAT® RM System and Cultibag RM 600 in any way. Modification may affect the safety of device.

3.4.2 Surface temperature sensor, plug in

The surface temperature sensor is mounted at rocking table.
Connection cables have to be plugged in.



1. Correct placement means the surface of the sensor is visible on top so that it is in direct contact with the CultiBag RM 600 film as shown in the picture.



2. Guide connection ribbon cables with plug through the opening of rocking table at the front side.

NOTICE

Incorrect placement of the temperature sensor can cause overheating of the heater plate and the bimetal will disconnect the thermal energy after reaching 50 – 60°C.

3.4.3 Filter heater, plug in

Two filter heaters are supplied in individual boxes. Connection cables have to be plugged in for operation. Both filter heaters are identical and are operated only while the gasflow is supplied into the bag.

1. Place both filter heaters at the lid of the rocking table with corresponding magnets.

2. Guide the connection cable with plug through the cable port on the lid.

3. Plug in the LEMO plug of filter heaters into the BIOSTAT® CultiBag RM 600 as shown in the picture.

3.4.4 Aeration Tube

An Aeration tube is included with delivery to connect the air in filter of CultiBag RM 600 with the BIOSTAT® RM Control Tower.

1. Place the aeration tube on the rocking table.

2. Guide the tube with plugs through the opening of rocking table at front side.

3. Connect the tube into the corresponding tube connectors at left side of BIOSTAT® CultiBag Control Tower.

3.4.5 Connect Optical Fibre Cables

Two optical fibre cables are included with delivery to connect CultiBag RM 600L with BIOSTAT® RM Control Tower.

1. Place both optical fibers cables on the rocking table.

2. Guide both optical fibers cable through the opening of rocking table at front side.

3. Connect the light fiber cables into the corresponding sockets at the rear side of BIOSTAT® RM Control Tower.
   Please refer to the associated manuals for Control Tower.

3.4.6 Connect Power Supply

1. Connect your power supply with BIOSTAT® CultiBag RM 600 system. Also please refer to the sections on Power supply and Mains connection.

2. Connect your power supply with BIOSTAT® RM Control Tower.
   Also please refer to the sections on Power supply and Mains connection.

3.4.7 Connect Pressurized Air, O_2, N_2 and CO_2 Supply

1. Connect the gas supply lines to the gas inlet ports on the of BIOSTAT® RM Control Tower 600.
   All gas types (Air, O_2, N_2, CO_2) need to be filtered and the inlet pressure reduced to 1.5 bar g.
   Please refer to the associated manuals for Control Tower.

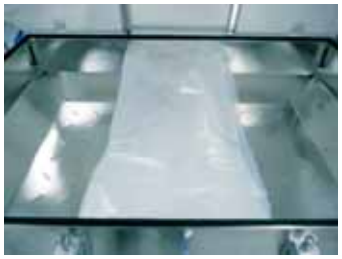
4. Preparation and Inflation of the CultiBag RM 600

4.1 Unpacking the CultiBag RM 600

NOTICE

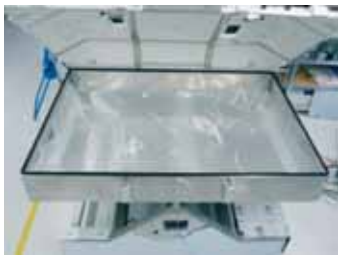
Risk of damaging the sterile cultivation chamber!

Remove the outer protective plastic film carefully. Take extra care when using sharp objects that may puncture the bag film.



Place the sterile disposable CultiBag RM 600L bioreactor chamber in the middle of the rocking table before removing the protective plastic foil!

Remove the sterile disposable CultiBag RM 600L bioreactor from the protective plastic foil as delivered.



Place the sterile disposable CultiBag RM 600L bioreactor chamber on the rocking table.

4.2 Mounting the CultiBag RM 600L on the Rocking Table

NOTICE

Make sure, the heating sections are not switched on before touching the rocking table. Rocking table might be hot.

Do not use any tools to mount the CultiBag RM to avoid damaging the bag.

Avoid any folds at the lower side of bag.

Do not start rocking motion while the cover of rocking table is opened. Risk of injury.

Check visually that the bag is properly installed. Install the bag rods completely in the guides.

Insert the both bag holders into the mount on the left and right side of the CultiBag RM 600L. Please avoid any damage at CultiBag RM 600L while fitting it on the rocking table!

Click the two holders into the corresponding lock on both site of the rocking table.

Inflate the CultiBag RM 600L until the air outlet filter has turned into an upright position.

NOTICE

Do not inflate the CultiBag RM 600L to the maximum pressure until the lid is closed safely.

NOTICE

Risk of damaging the sterile cultivation chamber!

Remove the outer protective plastic film carefully. Take extra care when using sharp objects that may puncture the bag film.

4.3 Installing the Filter Heaters

Place the sterile gas outlet filter inside the filter heater unit. (The air outlet filter is positioned on the left side of the bag). The proportional valve is mounted on the back side of the air outlet supporting plate.

If the gas outlet filters are equipped with a tube extension with mounted female lure coupling, please remove the tube extension and the lure coupling, then connect the filter outlet to the delivered tube with a 90°-bend, as showed in the figure.

After the 90°-bend and turn the tubing lightly downwards.

The vertical part of the tube after gas outlet filter should be embedded in the filter heater as much as possible.

It is necessary to use two gas outlet filters during aeration, so that the back pressure can be reduced through the outlet filters accordingly.

Both ends of the air outlet tubes are connected with one Y-piece. Connect the outlet of the Y-piece to the inlet of the proportional valve for pressure control as showed in the figure.

Please take care as the proportional valve can be very hot during operation.

Check the exhaust gas assembly, that there are no kinks or blockages in airflow in the tubing.

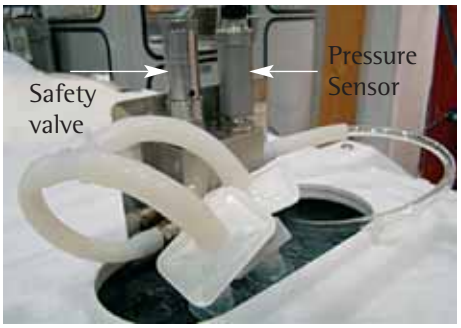
The both parts of the filter heater are connected by magnets, which are positioned at edge of the filter heater inside.
Place the filter heater box at the magnet surface of supporting plate.

Filter heaters are automatically switched on as soon as the gassing system of the BIOSTAT® CultiBag RM 600 is switched on. The air outlet filter will be heated up >40°C to avoid filter blocking.

NOTICE

Never run a cultivation without having mounted filter heaters at air outlet filter.

4.4 Connect Air Supply



Connect tubing to the gas outlet port of the BIOSTAT RM Control Tower and connect that to the inlet port of the measurement and safety valve, which is mounted on the right side of the air inlet supporting plate.

Connect the pressure sensor by using the delivered cable with M12 plug.

Use a Y-piece to connect two gas inlet filters to the gas outlet from the measurement and safety valve at the same time. This will reduce the backpressure through the gas inlet filters to a suitable range.

Check the gas inlet for any kinks or blockages in the tubing which may inhibit gasflow.

CultiBag RM 600L has five identical air filters mounted.
Three of them are grouped together (= air outlet filter).
Two filters are grouped together (= air inlet filters).
- Connect the air supply from the BIOSTAT® RM Control Tower with the air filters using the tubes delivered.
- Leave the air outlet filters open to allow air to leave the bag.

NOTICE

Direct connection to a gas source or use of any other air pump may cause overpressure, rupture the bag and void the warranty. The inbuilt pressure transmitter cannot feedback to an external source to stop aeration if overpressure is reached.

When there is no gas and liquid supply into the bag, we suggest clamping the tube between bag and gas outlet filters to avoid the filter blocking.

4.5 Inflation of the CultiBag RM 600

Please ensure the both inlet and outlet filter tubing clamps are open.

1. Switch on the main switch at front side of the rocking unit, the start menu will appear on the LCD-screen for a few seconds which shows the program version the system is working with. The display will then change to show the main menu.

NOTICE

To inflate the Cultibag RM we recommend to activate the pressure control loop and gas flow control loop in that order.

Please refer also to the section Over Pressure Management.

2. Allow the CultiBag RM 600 to fully inflate and ensure it is secured by the bag holder of to the left- and right side.

   The CultiBag RM 600 should be inflated firmly without creases.
   Ensure the air outlet filter is working by pressing down gently on the bag chamber and observing a release of air through the outlet air filter and proportional valve.
   Once the CultiBag is inflated reduce air flow to process values.
   Please refer to the section Set cultivation parameters and start.

4.6 Connect Optical Sensors

Observe the colour markers at the device, the aluminium clamp and the markers at the ends of the light fibre cables.
The pH has a yellow sticker, the DO has a red sticker. Do not mix up the light fibre cables.
Both ports are marked with a label with the inscriptions „pH“ and „DO“. Put the clamps on both ports of the pH und DO measurement.
The colours of the label have to comply with the colour of the respective light fibre cable i.e. red to red for DO and yellow to yellow for pH.

1. CultiBag RM 600 has to be inflated. It will not be possible to insert the optical fibre cables into the sensors ports of a CultiBag RM 600 that is not inflated.

   Put the clamps on both ports of the pH und DO measurement.

2. Insert the optical fibre cables into the sensors ports on the bag.
   Insert the optical fibre cable of DO measurement into port with red label “OptiSens DO.

   Insert the optical fibre cable of pH measurement into port with yellow label “OptiSens pH”.

3. The optical fibre cables are inserted until they touch the inner surface of the sensor caps. If there is no contact between the light fibre cable and the sensor cap no signal will be measured. Tighten the screws of the clamps to fix the cables in place.

4. For accuracy and for validated processes, the pH should be calibrated with an additional reference measurement. A sample of the media has to be taken asceptically from the bag and the offline pH and DO should be measured.

5. Cultivation

5.1 Preparing for the Cultivation

5.1.1 Filling the CultiBag RM 600 with culture media

For information regarding use of the intuitive Touch screen, please refer to the associated manual for BIOSTAT® RM Control Tower.

The CultiBag RM 600 should be inflated prior to filling with media, see section Inflation of the CultiBag RM 600. This will reduce creases and reduce foaming. Continue air flow and activate the pressure control loop to the headspace to keep the bag rigidly inflated, whilst filling with media.
Do not rock while filling.

1. Aseptically, connect tubing from the media container to the CultiBag RM 600 chamber using BioWelder, BioSealer, luer fittings or MPC couplings to connect two tubes together.

Guide the tubes through the openings on the left and right sides of the rocking table.

Use of luer and MPC couplings may require moving the CultiBag RM 600 Bag into a bio safety laminar flow cabinet for aseptic connection. The sampling luer may also be used.

2. Pump the desired volume of media into the bioreactor, and then aseptically disconnect the CultiBag RM 600 from the media container.

Do not use any tools to connect the CultiBag RM to avoid damaging the bag or tubes.

Avoid any folds at the tubes and do not squeeze the tubes.

Do not start rocking motion while the lid of the Rocker is opened. Risk of injury.

Ensure that both valves mounted at inlet and outlet filter are open. Also ensure the filter heaters are mounted correctly.
If the valves of the air filters are closed or are not connected while filling the bag with media, may result in overpressure inside the bag causing rupture of the bag.

3. Close the lid and lock all closures.

5.1.2 Set cultivation parameters and start

For information regarding use of the intuitive Touch screen, please refer to the associated manual for BIOSTAT® RM Control Tower.

1. Adjust required aeration rate. Start aeration if aeration is not already activated.
   For minimum and maximum aeration rate see section Aeration control and Aeration Flow rate.

   When using low or middle gassing rate (below 20 lpm), we recommend activating the pressure control loop to hold a constant middle pressure in the bag.
   When using high gassing rate (20 lpm or higher) the pressure control can be deactivated, because the gas outlet filter and the proportional will produce a back pressure in the bag.

   We recommend starting the aeration process parameter first, so that the gas outlet filters are preheated.

2. Adjust the rocking rate and rocking angle. A visible wave on the surface of the liquid is required. Reduce the speed if you see excessive foaming. Make sure that the bioreactor chamber is rigidly inflated; otherwise excessive foam will be generated from turbulence caused by creases in the bag.
   Start rocking motion.
   For preliminary estimation of rocking rate and rocking angle, see the section Optimize Wave Motion.
   For minimum and maximum rocking rate see section Rocking rate, for minimum and maximum rocking angle see section Rocking angle.

Always close the lid while rocking motion is activated.
Never put any objects on cover of rocking table while rocking motion is activated.
Avoid placing hands in the danger area of moving parts.

3. Adjust required set temperature.
   For minimum and maximum temperature see section Temperature control.

Do not start heating unless the surface temperature sensor is correctly positioned under the CultiBag RM. Otherwise over heating may occur.
Note that, if the rocking motion is started before you activate the heater, this will guarantee uniform temperature distribution in the medium.

5.2 Sampling and Inoculation

This operation requires a standard luer type syringe, whose luer septum is without hypodermic needle. It is not necessary to perform this procedure in a laminar flow cabinet.

5.2.1 Preparing for Sampling or Inoculation

For information regarding use of the intuitive Touch screen, please refer to the associated manual for BIOSTAT® RM Control Tower.

1. Continue headspace aeration.

2. Move the front side of rocking table down by adjusting the angle to keep the dip tube of the sample port in the media.
   Set angle in menu.
   For minimum and maximum of adjustable angle see section Rocking angle.

Do not open lid of rocking table.

3. Remove the protective cap from the luer septum sampling connector.

4. Disinfect the septum: wipe or spray the top of the Luer septum with 70% alcohol (or equivalent).

5. Connect the syringe: Using aseptic technique, attach a sterile disposable needleless syringe onto the luer connector.

6. Open the hose clamp of the luer septum. Draw the sample, close the hose clamp of the luer septum and the remove the syringe carefully.

5.2.2 Inoculation

For information regarding use of the intuitive Touch screen, please refer to the associated manual for BIOSTAT® RM Control Tower.

1. Inoculate the contents of the syringe via the Luer-Septum into the CultiBag RM 600.

2. Disinfect the Luer-Septum again and close it with the protective cap.

3. Drain back the liquid in the tubing.
   Pinch the sampling connector tubing a few times to ensure that any liquid in the tubing drains back into the bioreactor. Close the tubing clamp.

4. Restart the Rocking Motion
   Adjust the speed to get a visible wave on the surface of the liquid.
   Reduce the speed if you see excessive foaming.

Inoculation can also be done by any other port of the CultiBag RM 600.
Luer couplings and MPC couplings with their surfaces in contact with atmosphere have to connected in a laminar flow.
Use of BioWelder or BioSealer allows simple, fast and reliable connections .

5.2.3 Sampling

The procedure is the same as that described for inoculation using a needle less syringe.

The Luer septum is proven for 50 sterile samplings or inoculations. The connector contains a silicone septum, which automatically seals the device whenever it is disconnected.
The sample is also completely contained as no aerosols are vented. At low rocking rates (< 8 rocks/min.) the cells might settle in the media, which does not provide a representative sample. In this situation, the recommended technique is to raise the rocking rate more than 8 rocks/min., 5 up to 10 minutes prior to sampling. The rocking speed can be decreased after sampling.

The sampling device has a fairly small orifice. If you are working with large microcarriers or large cell aggregates do not use the sampling connector.

5.3 Media exchange

For information regarding use of the intuitive Touch screen, please refer to the associated manual for BIOSTAT® RM Control Tower.

1. Stop the rocking motion.
   The rocking table will stop automatically at horizontal position.

2. Clamp off the inlet and outlet filters.

3. Move front side of rocking table down by adjusting the angle to 0.

   Do not open the lid of rocking table.

3. Allow the cells or microcarriers 10 to 15 minutes to settle.

4. Change the culture media:
   Connect tubing to the C-Flex line at the front fill line (without dip tube).
   The other end of this tubing should be connected to a sterile collection vessel.
   Using a peristaltic pump, remove the desired amount of supernatant culture liquid by manually manipulating the flexible bioreactor wall (if required).
   Then disconnect the tubing and reconnect to fresh media to refill the bioreactor.
   Open the inlet and outlet filter clamps and restart aeration and rocking motion.

To avoid possible oxygen depletion, this entire operation should be performed in less than one hour.

6. End of the process and putting out of service safely

For information regarding use of the intuitive Touch screen, please refer to the associated manual for BIOSTAT® RM Control Tower.

1. Stop the rocking motion in main menu.
   The rocking table will stop automatically at the horizontal position.

2. Switch heaters off.

3. Stop aeration to interrupt air flow into the CultiBag RM 600.

   Do not open lid of rocking table.

4. Clamp off the air inlet and outlet filters.

5. Move the front side of the rocking table up by adjusting the angle to 0.
   For minimum and maximum of adjustable angle see section Rocking angle.

6. To drain all the contents via the harvest port (with dip tube at rear side) use a suitable peristaltic pump to pump out the contents, lift the bag up vertically for complete drainage.

7. To drain supernatant, follow procedure as per section Media exchange. Pump out the supernatant and add fresh media or transfer cell slurry into a larger CultiBag RM 600 reactor.

8. Store the CultiBag RM 600 under required process conditions before downstream processing.

9. Remove the air supply tubing from the air inlet filter.

10. Switch off pressurized air supply (if connected).

11. Switch off the power.

7. Cleaning and Maintenance Work

7.1 Intermediate Cleaning

The BIOSTAT® CultiBag RM 600 is a disposable bioreactor system so does not require cleaning and sterilizing like a conventional re-usable bioreactor. However, due to unavoidable spillages and associated aseptic techniques which are required for day to day operation, the equipment should be cleaned periodically:

1. The BIOSTAT® CultiBag RM 600 equipment can be cleaned using alcohol or commonly applicable detergents. Make sure the holder, which fixes the surface temperature sensor to the rocking table is not removed from the holder.

2. Disconnect power supply first. Wipe the housing of the BIOSTAT® CultiBag RM 600 with a wet cloth. Use water or common disinfection solutions such as 70% Ethanol. Do not use solvents | liquids which may damage or dissolve the materials of construction.
   Do not wash or submerge the equipment in liquid. Use only a wet cloth.
   Avoid liquid entering the housing. Major damage can be caused to electrical equipment by liquids entering the housing.

7.2 Maintenance by operators

Maintenance by operators is restricted to:

☐ Replace aeration tubing connecting the "AIR OUT" port of BIOSTAT® RM Control Tower and the sterile air in filter of CultiBag.

☐ There is no periodic lubrication required.

☐ Replace defective parts immediately such as:

Filter heater
Surface temperature sensor

☐ Service and calibration of BIOSTAT® CultiBag RM 600 is restricted to qualified service personnel, trained and authorized to do so.

7.3 Maintenance and calibration by authorized personnel

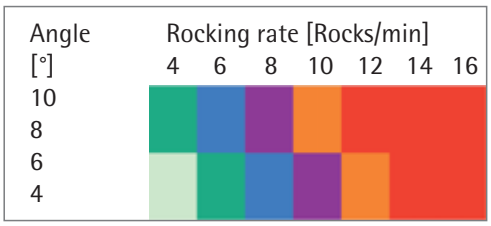
Some components such as bearings require regular maintenance.
The maintenance work is recommended every 4'000 working hours, this includes necessary calibration of all parameters.
The battery powering the PLC built-in in the Rocker 600 has a life time longer than five years. It is recommended to replace this battery within five years.
The maintenance work includes the replacement of battery if required.

Service and calibration of BIOSTAT® CultiBag RM 600 is restricted to qualified service personnel, trained and authorized to do so.
Please contact your local Sartorius Stedim Biotech service engineer for more information.

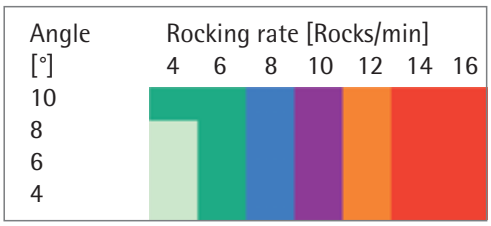
8. Supplement

8.1 Optimize Wave Motion

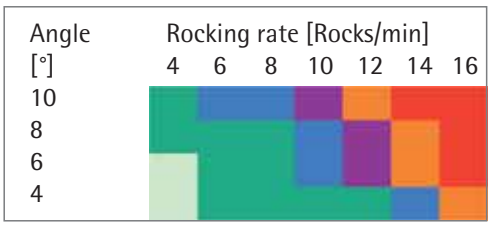
Every cell line requires optimization of operating conditions. The attached graphs reflect a guide line. Tests estimating optimum formation of wave mixing are required.
Specification of the CultiBag RM 600L optical:
Min. working volume: 60 L
Max. working volume: 300 L



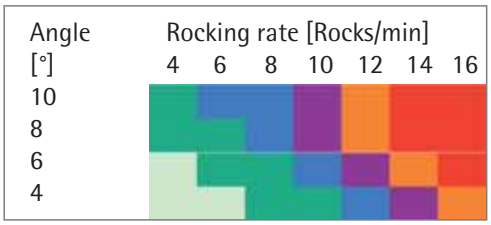
CultiBag RM 600L optical:
Working volume: 60L



CultiBag RM 600L optical:
Working volume: 150L



CultiBag RM 600L optical:
Working volume: 240L



CultiBag RM 600L optical:
Working volume: 300L

Legend

- Minimal wave forces, below limit.
  Minimal oxygen transfer, no shear forces
- Low wave formation, no forming, minimal wave forces.
  Low oxygen transfer, minimal shear forces
- Ideal wave formation, no framing, minimal shear forces.
  Well oxygen transfer, minimal shear forces
- Well wave formation, slight foaming, small shear forces.
  Very well oxygen transfer, small shear forces
- Heavy wave formation with foaming, middle shear forces.
  Very well oxygen transfer, heavy shear forces
- To heavy wave formation, heavy foaming, heavy shear forces.
  Very well oxygen transfer, to heavy shear forces

8.2 Over Pressure Management

A pressure transmitter monitors the feedback pressure from the CultiBag RM 600L. In case of overpressure above +45 mbar ± 5 mbar for more than 3 minutes, the metering valve will close until pressure drops less than +45 mbar ± 5 mbar. Thereby providing additional culture safety and bag seal integrity.

8.3 List of Alarm Messages

The DCU control system BIOSTAT® RM Control Tower provides standard features for alarm monitoring for process values and digital inputs.
Those standard features are described in the DCU operators manual.
This chapter describes only additional functions relating to alarms.

Process Value Alarms
The process values will be monitored for high and low limits.
The alarm indication will be performed with standardized text, e.g. "Process Value TEMP high alarm".
The operator can change the alarm limits and can enable or disable each alarm via the touch panel, given appropriate security access.
Please see also associated manuals for Control Tower.

System Power Failure
In case of power failure, the control tower keeps all controller states and setpoints within the FAIL TIME saved. The FAIL TIME alarm can be triggered via following operation:

Set the FAIL TIME [FTIME]. Disconnect the mains supply.
- If power supply comes back within the fail time, the system will reboot and all controllers will be still in the modes established before the reset, Alarm message "Power failure" will appear.

- If the power supply comes back after the fail time, the system will reboot and all controllers will be in "OFF" Mode, Alarm message "Power failure, Fermentation Stopped" will appear.

- In both cases the alarm message "System Power failure" will appear.
  The default setting for the fail time is 2:00 hours

Motor Safety Stop Alarm
Alarm event from Rocker which is connected via a serial interface. The operator cannot disable alarm, system restart is necessary.

Overpressure ALarm
See Chapter 8.3.
The operator can only disable this alarm, if the pressure returns to a normal operating pressure situation.

8.4 Trouble Shooting Disturbances and Measures

Frequently Asked Questions. For further information contact your local Sartorius Stedim Biotech representative.

Disturbance	Possible causes	Measures
Bag appears to be over inflated.	The CultiBag RM 600 should be inflated firmly without creases. Nevertheless it shouldn't be so full that it is over pressurized and creates hinges at the fixing points.	If the bag is over inflated, check if there is air leaving the sterile air outlet filter. Connect the filter with a piece of hose and hold it in water filled water glass. If you cannot see bubbles the air outlet filter is closed.
Bag appears to be under inflated.	If the CultiBag RM 600 is under inflated, it creates excessive foaming and bad mixing. "No airflow" alarm may be shown on display.	Check the AIR OUT port of BIOSTAT® RM Control Tower to ensure air is being released. The sterile air inlet filter may be clogged, slide clamp may be closed, or air supply tube may not be properly fixed. Use a spare filter as inlet or transfer your culture medium into a new bag.
Excessive foam in CultiBag RM 600.	A certain amount of foam is typical for the BIOSTAT® CultiBag RM 600. But the foam shouldn't cover more than 50% of the medium surface.	If too much foam occurs check if the CultiBag RM 600 is correctly inflated. Under inflation generates a lot of foam. Usually foam will disappear after a number of hours. If then the foam is still too high reduce the rocking rate. Check that the reduced rocking rate is high enough to guarantee a sufficient dissolved oxygen concentration in the media. If foam is still a problem reduce the rocking angle. The reduced rocking angle generates less foam and the rocking velocity can be enhanced again a little bit. For further information, contact your local Sartorius Stedim Biotech representative.
Bioreactor heats too slowly.	Media is not heating up or is heating very slowly.	Make sure that surface temperature sensor is correctly positioned under the bag. Check that the set point is set correctly. Verify that BIOSTAT® CultiBag RM 600 is running in correct mode, see section Characteristics, Specification of CultiBags RM, operation modes RM Control Tower . Check that BIOSTAT® CultiBag RM 600 is rocking. Remember that the heater is intended to control temperature using gentle heating. To heat up 300 liters from ambient temperature to 37°C will take several hours.

Disturbance	Possible causes	Measures
Temperature control not accurately	Temperature of media is not controlled accurately.	Ensure that the surface temperature sensor is correctly positioned under the bag. Check that BIOSTAT® CultiBag RM 600 is rocking. If the temperature is still not controlled properly, recalibration of temperature measurement might be required. Contact your local Sartorius Stedim Biotech representative.
Temperature appears to be incorrect	Actual temperature shown in main menu does not correspond with independent measurement or ambient temperature.	Make sure that surface temperature sensor is correctly po¬sitioned under the bag. Check that BIOSTAT® CultiBag RM 600 is rocking. If actual tempera-ture is still not correct, recalibration of temperature measurement might be required. Contact your local Sartorius Stedim Biotech representative.

8.5 Provisions

8.5.1 Warranty Aggreements and Intended Use

All components of the BIOSTAT® CultiBag RM 600 are designed for biotechnological processes commonly applied in a corresponding laboratory environment. If questions arise, e.g. at use of aggressive media (such as corrosive media) with the CultiBag RM or in the environment you will have to test the corrosion resistance of all components.

All products of Sartorius Stedim Systems have a warranty in accordance with the General Terms and Conditions of Business, unless other terms were agreed upon in writing.

- ☐ The warranty is granted against defects of construction, manufacture or in material and malfunctioning resulting therefrom.
- – Faulty equipment will be repaired or replaced.
- ☐ The warranty does not refer to consumables and parts, which are subject to usual wear and tear (e.g CultiBags, Filterheater etc.).

The warranty will lapse:

- ☐ at erroneous handling of the bioreactor and its equipment, if they are applied beyond their specifications or accompanying instructions are ignored;
- ☐ at operation under unsuitable ambient conditions, e.g. at the presence of corrosive acting media;
- ☐ at use of components from third party suppliers, for which Sartorius Stedim Biotech has not confirmed that they can be used.

8.5.2 Service

Defective equipment cannot be repaired in a customers service shop.
Defective equipment can also be returned to Sartorius Stedim Biotech.
Repairs will be carried out in accordance with Sartorius Stedim Biotech' Terms of Maintenance. For return of equipment please contact local Sartorius Stedim Biotech Representative.

Biohazards possible. Risk of infections!
On return the equipment must be clean, in good hygienic condition and carefully packed. Contagious parts must be disinfected or sterilized, according to the safety regulations related to the application, for which the devices are used.

The sender has to prove compliance with the safety regulations.

A „Declaration about decontamination and cleaning of equipment",
which can be used for return of equipment, is attached below.

8.5.3 Disposal of Equipment

CultiBags are disposable and are discharged after each cultivation. Sterilization may be required before disposal of bag. Treatment within national regulation of disposable of biohardazous equipment.

1. Switch off the power. Disconnect from power supply.
2. Disconnect pressurized air supply.
3. Disinfect or decontaminate and clean contaminated devices if required.
4. Dismantle BIOSTAT® CultiBag RM 600 into its components:

☐ Stainless steel parts.
☐ Components made of plastic.
☐ Electrical components
☐ Electronical components
☐ Battery
☐ Observe the respective national regulations.

8.6 Decontamination Declaration

For return of faulty equipment to Sartorius Stedim Systems GmbH in case of warranty and for service the sender will have to enclose a decontamination declaration to the shipment. This declaration shall describe, which media, microorganisms or cells have come into contact with the equipment and what the sender has done for the disinfection or decontamination.

☐ The recipient (i.e the service of Sartorius Stedim Systems GmbH) must be able to see the decontamination declaration before unpacking.

☐ A form of the decontamination declaration for the return of equipment is attached below or available as separate document.
For additional copies of this document please contact Sartorius Stedim Systems.

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equipment without notice.

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